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Effect of rejuvenation on biochemical properties of fruit and fruit pulp during different storage period in Guava (*Psidium guajava*) CV Allahabad safeda

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Abstract
Rejuvenation of guava is one of the most important practices that influence the vigor, productivity and quality of the fruits (Gadgill and Gadgill, 1933). Guava is considered as one of the exquisite, nutritionally valuable and remunerative fruit crop. Guava bears on current season's growth and flowers appear in the axil of new leaves. Therefore, An experiment was laid out to study the biochemical response of rejuvenated old guava orchard cv *Allahabad Safeda* at the demonstration farm of Jawahar Lal Nehru Krishi Vishwa Vidyalaya, Krishi Vigyan Kendra, Seoni during 2009-10. Sixteen year old guava plants were pruned drastically leaving only four scaffold branches per tree at monthly interval from April 2009 to June 2009. In rejuvenated guava, the highest value of TSS 12.34 °Brix was observed when 100% yellow fruit was taken, whereas the lowest (10.89 °Brix) was observed in 100% green fruits. In unrejuvenated guava, the lowest TSS (10.71 °Brix) was observed when the fruits were harvested at 100% green and 11.67 °Brix when 100% yellow fruits were taken. Higher ascorbic acid content in fruits (141.82 and 163.11mg/ 100g, respectively in un-rejuvenated and rejuvenated plant) was recorded in 100% yellow fruits. Significantly higher percentage of free soluble sugar (11.30) was estimated in fruits obtained from rejuvenated trees as compared with control (10.65%). Analysis of the pulp showed that there was a slight increase in the pulp recovery extraction in rejuvenated guava fruit (12.07%) and did not show remarkable difference in total soluble soilds, moisture%, acid, ascorbic acid and pectin contents of pulp. Pruning proved to be successful in rejuvenating an old guava orchard of cv. Allahabad Safeda.

Keywords: guava, rejuvenation, pulp, ascorbic acid, orgenoleptic

Introduction
Guava (*Psidium guajava*) is one of the important commercial fruit crops for tropical & subtropical climatic regions of India. It has excellent digestive and nutritive value, pleasant flavour, high palatability and availability in abundance at moderate price. The major guava producing states in India are Uttar Pradesh, Maharashtra, Bihar, Andhra Pradesh, Gujarat, Madhya Pradesh, Karnataka, Punjab and Orissa. India is the leading producer of guava in the world. The total area and production of guava in India is about 268 thousand ha, 3997 thousand MT respectively (NHB 2016-17 to 2017-18 3rd Advance Est.). In Madhya Pradesh the total area, production and productivity of guava are 22.4 thousand hectare, 841.1 thousand million tonnes and 37.6 MT/ha respectively. Madhya Pradesh rank's 1st in productivity with 37.6 t/ha (NHB, 2013-14). In, Madhya Pradesh guava is cultivated some district Rewa, Satna, Balaghat, Panna, Seoni, Umaria, Katni, Sheopur, Gwalior, Shivpuri, Betul, Chhindwara, Guna, Ratlam, Ujjain, Jhabua, Dhar, Indore, Dewas etc.

A guava fruit is also termed as "Poor man's apple" because it is nutrient rich, cheap and easily available to the common man in the plains of Northern and Central India. Guava is one of the richest natural sources of vitamin – C containing 2 to 5 times more vitamin C than oranges and 10 times more than tomato. It is a rich source of calcium, phosphorous and iron which are necessary for human health. Guava fruits are used for both fresh consumption and processing. Guava is cultivated on large scale in Madhya Pradesh but the productivity of guava is low due to old and dense orchards. It has been observed that after 15 to 20 years of age guava orchards generally begin to lose vigour, bearing potential & poor fruit quality. Low productivity of guava is primarily due to small size of holdings, preponderance of old and senile orchard and poor management of inputs such as water nutrients and pesticide. Hence the present experiment was conducted to assess the physico-biochemical response of rejuvenation on old and senile orchard of guava.

Pruning is judicious removal of plant parts to establish balance between vegetative and productive growth. It has strong influence on fruitfulness of plants. Pruning had a significant effect on the guava tree height, tree spread and canopy volume was affected significantly by various pruning operations. Pruning trees to manageable size keeps them healthy and is necessary for optimal fruit production and longevity of the tree. Guava bears on current season shoots in the axils of leaves.

Materials and Methods

The field experiment was conducted to study the biochemical response of rejuvenated & unrejuvenated guava fruits at the Demonstration Farm of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Krishi Vigyan Kendra, Seoni during the year 2009-10. Sixteen year old guava plants were pruned drastically at monthly interval from April to June 2009 leaving only four scaffold branches per tree. The rejuvenation method involved the heading back of exhausted trees to the extent of 1.5 meter height above the ground level during April to June. The treatments consisted of rejuvenation of current season growth *i.e.* unrejuvenated, rejuvenated in the month of April, rejuvenated in the month of May and rejuvenated in the month of June at 7 m x7 m meter spacing guava tree. Each treatment was replicated thrice with single tree as experimental unit in randomized block design. The selected trees were maintained under uniform cultural practices. The fruiting characteristics of winter season crop were recorded from September to January. The data on quality characters of fruits were determined in terms of total soluble solids content of juice was determined with the help of Bausch and Lamb's hand refractometer in terms of degrees Brix. The value of total soluble solids was recorded at 20^0 C with the help of temperature correction chart (AOAC, 1970) [3].

Guava fruit harvest at different ripening stages

Harvest maturity of guava has been standardized based on visual appearance and destructive tests. There are a continuous decrease in specific gravity (SG), with ripe fruit reaching values <1.0. Skin color can be considered as a good maturity harvest index, due this index showed the majority of the correlated index in both cultivars. Skin color showed the best indicator to define the ripening stage for both unrejuvenated and rejuvenated guava plants studied. However, the fruits from unrejuvenated and rejuvenated guava plants were picked at different harvesting Stages 100% green, 60% Yellow, 80% Yellow and 100% Yellow stages and biochemical analysis was done during the same.

Determination of biochemical properties: Pulp: Seed ratio was calculated by weighing the flesh and its corresponding seed content separately. The flesh weight was divided by seed weight. True density was calculated by toluene displacement method and percentage moisture content was determined by gravimetric method (AOAC 1965) [2]. Total soluble solids was determined by a hand refractometer at 20^0C (range 0-50%) as per AOAC (1970) [3] method. Titrable acidity was determined as percent citric acid by titrating with 0.1 N NaOH using phenolphthalein as indicator and calculated as follows:

$$\text{Titrable acidity \%} = \frac{\frac{64/1000 \times \text{N. NaOH} \times \text{Titre} \times \text{Volume made up}}{100}}{\frac{\text{Volume of sample taken for estimation}}{\text{wt. of sample taken for estimation}}}$$

Ascorbic acid content was estimated by titrating the sample with 2-6-dichlorophenol-indophenol dye and calculated as per the formula given below:

$$\text{Ascorbic acid mg /100 g} = \frac{\frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot of extract Taken for estimation}}}{\text{Volume of sample taken for estimation}}$$

The above methods are briefly described by Ranganna (1986) [8]. Free soluble sugars were estimated by the procedure of Dubois *et al.* (1956) [4].

Extraction of pulp: Guava fruits were washed thoroughly with clean running water and cut into thin slices with a stainless-steel knife. Fruits slice mixed with 25% water and heated at 80ºC for 5 min to soften fruits slices. The soften fruits slices were then passed through using superfine pulper with 32 mesh size to obtain homogenized pulp without seeds. The pulp were filled in pre sterilized bottles of 200 ml capacity leaving 2.5-3.0 cm headspace, sealed with crown corks pasteurized in boiling water on a false bottom for 25 to 30 min. The pulp filled bottles were than cooled in air and stored at ambient temperature (30-35º C) for two month. The analytical parameter i.e. total soluble solid, moisture, titratable acidity, pH, ascorbic acid, reducing sugar, total sugar, pectin, and ash content were determined by Standard analytical method (AOAC 1970) [3]. The treatments were replicated thrice, and the data obtained were subjected to analysis of variance (ANOVA) using factorial completely randomized design as described by Panse and Sukhatme (1985) [7]. The critical difference value at 5% level was used for making comparison among different treatments and storage period.

Organoleptic quality Analysis: The organoleptic quality of products was evaluated by a panel of 10 judges on 9-point hedonic scale (Amerine *et al* 1965) [1]. Storage experiments of guava pulp were carried out for a period of 60 days and the quality analysis was made. The periodical observations on biochemical qualities were evaluated by opening three bottles from each storage condition randomly. Biochemical analysis were observed at every 30 days. The biochemical analysis included TSS, Acidity, total sugar, and ascorbic acid.

Results & Discussion

Only winter season crop was obtained one year after pruning and pruned plants flowered one month ahead of unpruned (control) plants. Time of Pruning had significant effect on fruit set. Fruit weight, total soluble solids, total sugars and Vitamin-C content were significantly improved by pruning. The fruit produced from pruned trees had better quality as compared to control. The results of this experiment provide insight into the interaction effect of rejuvenation pruning and cropping pattern on guava trees.

Biochemical properties

The variation of biochemical parameters with ripening is shown in Table -1. It was observed that the total soluble solids and ascorbic acid content in fruits increased with ripening stage. In rejuvenated guava, the highest value of 12.34 °Brix was observed when 100% yellow fruit was taken, whereas the lowest (10.89 °Brix) was observed in 100% green fruits. In unrejuvenated guava, the lowest TSS (10.71 °Brix) was observed when the fruits were harvested at 100% green and

11.67 °Brix when 100% yellow fruits were taken. This data indicating significant higher TSS value in 100% yellow fruits. A positive correlation was exists between pigmentation and other biochemical changes responsible for increasing the total soluble solids.

Titrable acidity decreased with ripening of rejuvenated and un-rejuvenated guava. Highest acidity (0.80%) was observed at 100% green stage of fruits and least of acidity (0.51%) was recorded in 100% yellow fruit stage in un-rejuvenated guava. In rejuvenated guava, 100% yellow fruits gradually decreased with the ripening of fruits up to 0.61. Higher ascorbic acid content in fruits (141.82 and 163.11mg/ 100g, respectively in un-rejuvenated and rejuvenated) was recorded in 100% yellow fruits. Ascorbic acid content in rejuvenated guava increased with the advancement of ripening of fruits in comparison to un-rejuvenated guava. Lowest ascorbic acid content among all the stage of maturity was observed in 100% green fruits of un-rejuvenated and rejuvenated guava (108.00 and 116.93 mg/100 g). Significantly higher percentage of free soluble sugar (11.30) was estimated in fruits obtained from rejuvenated trees as compared with control (10.65%). Free soluble sugar content differed from each other significantly with increased ripening of fruits. Flesh and seed ratio in fruits increased with advanced stages of ripening of rejuvenated and un-rejuvenated trees. In rejuvenated guava 3.5:1 (Flesh: Seed) were recorded as compared with un-rejuvenated guava (3.2:1) at 100% yellow stage.

Pulp analysis: In the present investigation result showed that there was a slight increase in the pulp recovery extraction, did not show remarkable difference in total soluble solids, moisture, acid, ascorbic acid and pectin contents of pulp. Moreover, the pulp recovery 68.00% and TSS 12.30 % more from rejuvenated guava and it was superior in flavor than unrejuvenated guava. The pectin content was not affected much with very minor changes thus showing non significant effects under unrejuvenated guava. Peeling losses were slightly higher in unrejuvenated Guava. The organoleptic quality assessed unrejuvenated and rejuvenated guava fruits pulp was found acceptable. Rejuvenated guava fruits pulp found better overall organoleptic mean score.

There was a considerable increase in total soluble solids of pulp during storage, which may be due to increase in sugars, acidity and other soluble materials. Tandon and Kalra (1984) [10] reported increase in total soluble solids of guava pulp during storage. The decrease in moisture content of pulp 87.79 % in 'Un-rejuvenated guava' and 85.80 % in rejuvenated guava. The acidity of pulp from both the method increased and the percent increase was greater in ' rejuvenated guava 'than in' Un-rejuvenated guava. The pH of both the method showed a downward trend during storage. The results of changes in acidity and pH are in conformity with those reported by Tandan *et al*. (1983) [11]. The reducing sugar contents of pulp increased remarkably during storage which might be due to the hydrolysis of polysaccharides like pectin and starch into simple sugars. Similar observations were also recorded by Roy and Singh (1979) [9] in bael products. There was a significant increase in reducing sugars in blends during storage. The total sugars increased in stored pulp, the extent of increase was more in ' rejuvenated guava ' than ' Un-rejuvenated guava ' which may be due to conversion of starch into sugars. Harnanan *et al*. (1980) [5] reported increase in both reducing and non-reducing sugars in stored guava pulp. There was significant decrease in ascorbic acid content of all the samples. Ascorbic acid is sensitive to heat and is oxidized quickly in the presence of oxygen (Mapson 1970) [6]. Hence, it might have been destroyed during processing and subsequently during storage period due to its oxidation.

Changes in chemical composition of guava pulp during storage at ambient condition

The observations of TSS, Moisture, Acidity, Reducing sugar, were recorded on 0, 30th and 60th days of storage. It was observed that, pulp from rejuvenated guava fruit stored at ambient storage condition had performed significantly higher over unrejuvenated guava fruit. Whereas, the TSS ranges between 11.65 to 12.93 % from unrejuvenated guava fruit pulp and 12.30 to 13.23 from rejuvenated guava fruit pulp were observed during 0 to 60 days of storage. Similarly moisture % were found 87.70 to 83.43 from unrejuvenated guava fruit pulp and 85.80 to 81.23 from rejuvenated guava fruit pulp. Acidity was found 0.48 to 0.55 from unrejuvenated guava fruit pulp and 0.55 to 0.58 % from rejuvenated guava fruit pulp. Reducing sugar and total sugar % ranges 3.32 to 5.35 and 10.60 to 10.90 from unrejuvenated guava fruit pulp and 3.55 to 5.63 and 11.30 to 11.67 from rejuvenated guava fruit pulp respectively. pH and Ascorbic acid content ranges 3.96 to 3.82 and 3.81 to 3.71 from unrejuvenated guava fruit pulp and 32.34 to 20.50 and 34.62 to 22.45 from rejuvenated guava fruit pulp respectively.

Evaluation of organoleptic qualities of unrejuvenated & rejuvenated guava fruit pulp

Table-4 shows the composite score of the fruit pulp in terms of Appearance, color, flavor, taste and overall acceptance. The overall acceptability was 7.17 in unrejuvenated guava fruit pulp and 7.40 was in rejuvenated guava fruit pulp after 60 days of storage However, the rejuvenated guava fruit pulp could retain the flavor to a greater extent. This might have influenced the overall acceptability for which remarkable differences could be observed. Storage study revealed that though there was a change in biochemical qualities of guava fruit pulp samples after 60 days of storage, the changes were less remarkable in rejuvenated guava fruit pulp storage as compared to unrejuvenated guava fruit pulp.

Table 1: Bio-chemical properties of fruits with respect to stage of ripening of unrejuvenated and rejuvenated guava fruits

Unrejuvenated Guava						Rejuvenated Guava				
Stage of harvest	**Flesh: Seed**	**TSS (°Brix)**	**Acidity %**	**Vitamin C (mg/100 g pulp)**	**Total sugar (%)**	**Flesh: Seed**	**TSS (°Brix)**	**Acidity %**	**Vitamin C (mg/100 g pulp)**	**Total sugar (%)**
100% Green	2.3:1	10.71	0.80	108.00	7.05	2.6:1	10.89	0.71	116.93	9.22
60% Yellow	2.4:1	11.12	0.73	118.94	8.03	2.8:1	11.81	0.67	141.58	10.18
80% Yellow	2.8:1	11.54	0.67	135.16	9.09	3.1:1	12.07	0.65	154.34	10.76
100% Yellow	3.2:1	11.67	0.51	141.82	10.65	3.5:1	12.34	0.61	163.11	11.30
CD (P=0.05)	-	NS	0.07	16.44	0.93	-	1.06	NS	13.08	0.98

Table 2: Composition of guava pulp of unrejuvenated & rejuvenated guava fruit

Parameter	Unrejuvenated Guava Pulp	Rejuvenated Guava pulp
Total soluble solids, %	11.65	12.30
Moisture, %	87.79	85.80
Acidity, %	0.48	0.55
Reducing Sugar %	3.32	3.55
Total sugars, %	10.60	11.30
Ascorbic acid, mg /100g	140.42	158.23
Pectin, %	0.42	0.67
pH	3.97	3.81
Ash, %	0.70	0.49
Pulp recovery, %	58.30	68.00
Peeling losses, %	6.6	5.30

Table 3: Changes in chemical composition of guava pulp during storage at ambient condition.

Treatment	Unrejuvenated Guava Pulp			Rejuvenated Guava Pulp		
	0 days	30 days	60 days	0 days	30 days	60 days
Total Soluble Solid, %	11.65	12.10	12.93	12.30	12.80	13.23
Moisture, %	87.70	85.12	83.43	85.80	83.45	81.23
Acidity, %	0.48	0.52	0.55	0.55	0.58	.0.58
Reducing Sugars, %	3.32	4.45	5.35	3.55	4.42	5.63
Total Sugars, %	10.60	10.72	10.90	11.30	11.45	11.67
pH	3.96	3.91	3.82	3.81	3.78	3.71
Ascorbic acid mg/100g pulp	32.34	25.23	20.50	34.62	28.67	22.45
Overall acceptability	8.34	7.64	6.99	8.60	8.34	7.89

Table 4: Evaluation of organoleptic qualities of unrejuvenated & rejuvenated guava fruit pulp.

Treatment	Unrejuvenated Guava pulp	Rejuvenated Guava pulp
Appearance	7.67	7.87
Colour	7.56	7.56
Flavour	7.14	7.54
Taste	7.07	7.67
Overall acceptance	7.17	7.40

Conclusion

From this experiment it may be concluded that time of Pruning had significant effect on biochemical properties of fruits and fruit pulp *viz*. Total soluble solids (TSS), Titratable acidity, Total sugars and Vitamin-C content were significantly improved by pruning. The fruits obtained from pruned trees had better quality as compared to control. Ascorbic acid content in rejuvenated guava increased with the advancement of ripening of fruits in comparison to un-rejuvenated guava. The yield and quality of pulp from rejuvenated was better than un-rejuvenated Guava fruits. The organoleptic quality assessed unrejuvenated and rejuvenated guava fruits pulp was found acceptable. The rejuvenated and unrejuvenated plant guava fruit pulp of sample stored at ambient storage condition were acceptable up to 60 days. However, the pulp samples stored of rejuvenated guava pulp was found superior over unrejuvenated plant guava fruit pulp. Results of study concluded that rejuvenated guava fruit was found superior as compared unrejuvenated plant guava fruit.

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